Chemical Crosslinking - Probing the interface of proteins

Proteins are essential components of organisms and are necessary for the function of virtually every process within cells. Because of the importance of proteins, a lot of research aims to determine protein structure and protein-protein interactions. To date, the most popular techniques for this work are high resolutions methods such as x-ray crystallography and nuclear magnetic resonance spectroscopy (NMR). However, these techniques have limitations. They required large amounts (10-100mg) of very pure protein. NMR is also limited by the size of the protein (~40kD limit), while crystallography relies on the formation of a crystal.

Chemical cross-linking is a technique that has been used for a long time to study protein-protein interactions; however, it has gained popularity more recently upon the coupling of cross-linking with mass spectrometry. In this technique, proteins are chemically cross-linked through reacting them with a cross-linker reagent, which introduces a covalent bond between specific amino acids of the protein. The cross-linker imposes a distance constraint on the location of protein functional groups capturing a snapshot of their interaction. This technique can be used intramolecularly to gain insight into the 3D structure of a single protein as well. Some of the major advantages to cross-linking are: it is applicable to low (µM) concentrations of protein; it captures a snapshot of the protein in its native/dynamic conditions; it can be easily coupled to mass spectrometry for detection of cross-linked products and identification of cross-link site.

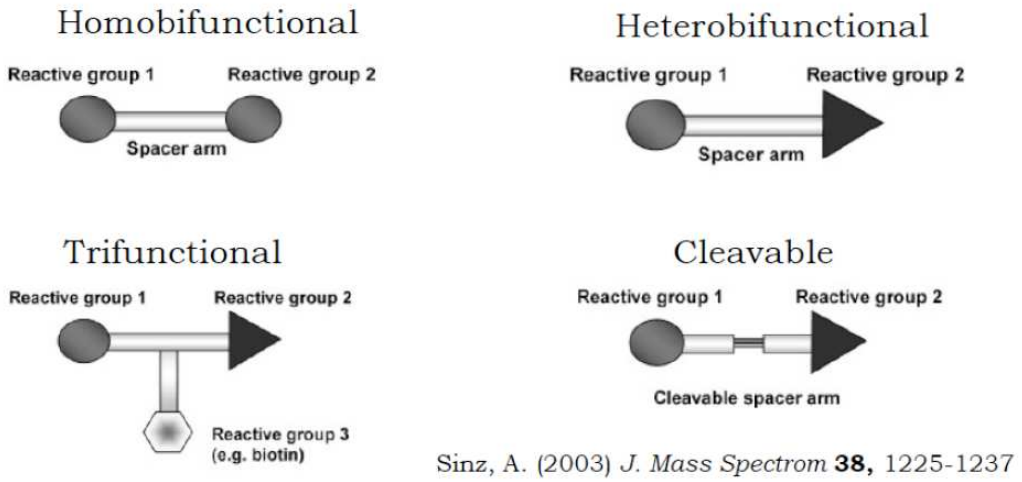


Figure 1: Examples of common cross-linker design

The experimental protocol used for a typical cross-linking experiment is outlined below. The proteins are of interest are combined in solution with the cross-linking reagent and allowed to react for a certain period of time. Cross-linked product is confirmed with an SDS-PAGE gel before in-solution digest with trypsin. Analysis of cross-linked reaction mixture is performed using mass spectrometry. An isotope labeled (d_4) version of the cross-linker reagent (BS^2G) is used to help identify the cross-linked products by searching for the d_0/d_4 pattern in the mass spectra.

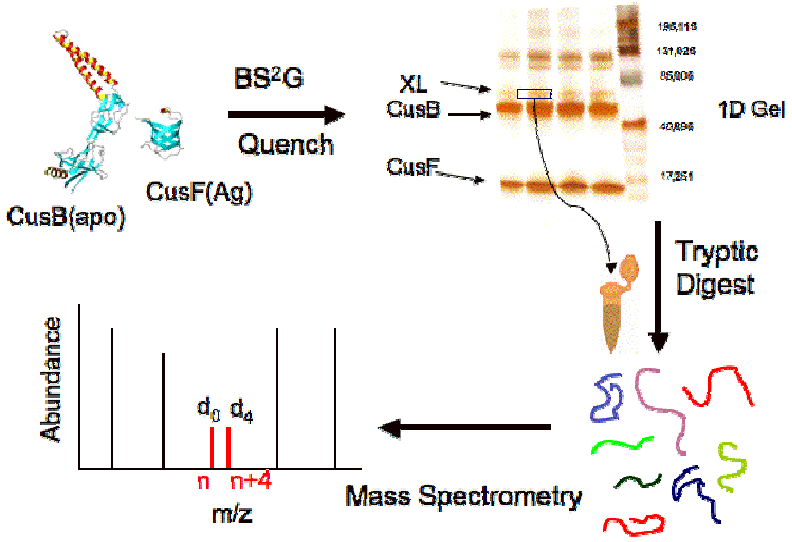


Figure 2: Protocol for cross-linking experiment

Different protocols might apply on the details of how the crosslinking reaction is carried out. Essentially, crosslinkers are added into the proteins that are interacting with other proteins. And the crosslinkers are supposed to capture the interactions by forming covalent chemical bonds with accessible amino acid residue sidechains on the proteins. Then after a certain amount of time, the proteins are enzymatically digested directly in solution or in gel bands after separation. By analyzing the digest on a mass spectrometer (usually with LC separation), the peptides that are crosslinked can be detected because they have modified mass due to the crosslinker. With good quality MS/MS spectra and pre-knowledge of which amino acid sidechains the crosslinker reacts with, the exact amino acid residues that are crosslinked could be determined. This step is often more efficiently done with computer algorithms than manual processing because they are too many possibilities for two pair of peptide to be crosslinked. There are programs available for this purpose, such as *ProteinXXX* and *Popitam*. And people in the group also came up with customized programs to process experimental data.

It can be then determined that which residues in the protein are crosslinked to each other from the MS data. In the protein complex structure, these residues should be apart from each other at the distance of the length of the crosslinker used in the study. This spatial constraint information can be combined with mutation study and structures from X-ray crystallography or NMR to further examine the biological significance of the binding interface. Additionally, computational docking structure of the proteins offers theoretical insight that builds up the confidence in the result. The docking structure can be calculated with molecular dynamics from the experimental crystal structure of individual proteins. Results of LexA and RecA crosslinking showed good

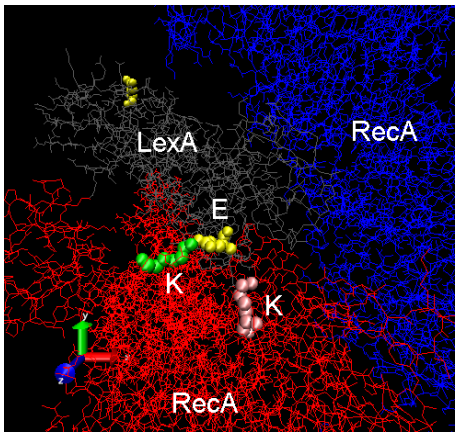
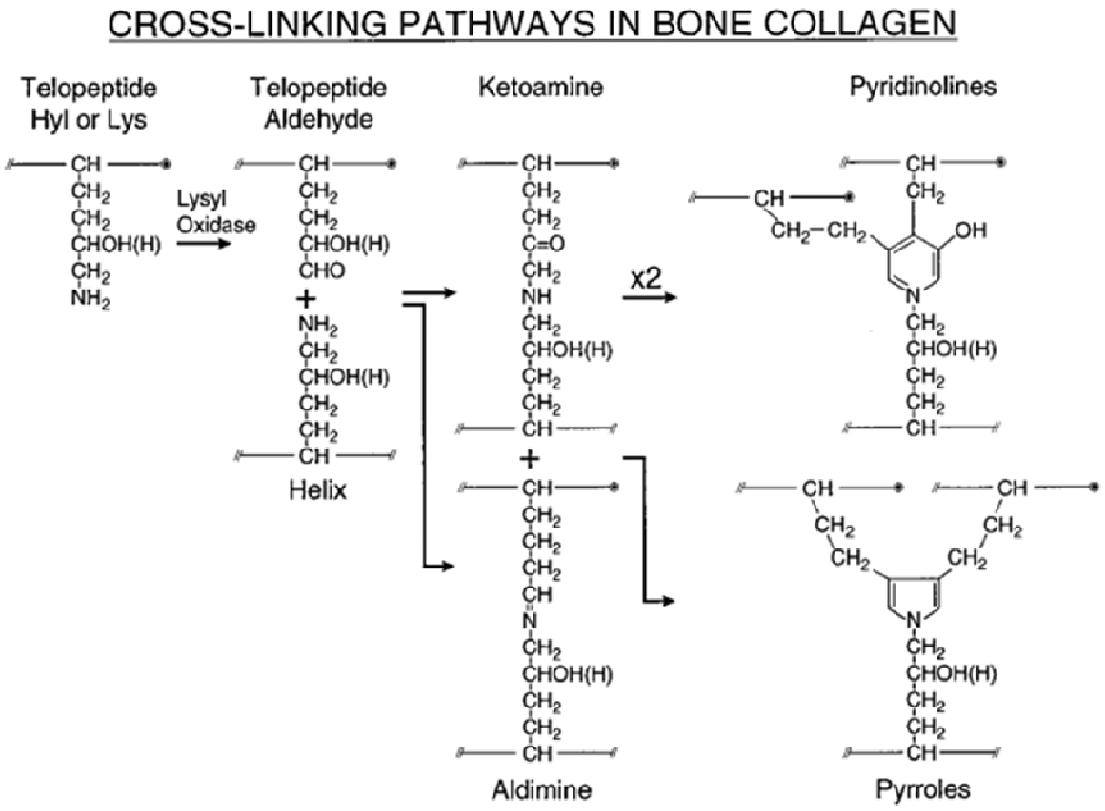


Figure 3: K (green) and K (pink) are experimentally found to be crosslinked with E (yellow).

consistency with the docking simulation and experimental results (***Fig 3***). The amino acid residue pairs that are found to be close to each other in the crosslinking experiment are also in proximity in the simulated structure.

Collagen Crosslinking

We are also interested in studying endogenous crosslinks of collagen in collaboration with Douglas F. Larson in the Sarver Heart Center. Our hypothesis is that hyper-crosslinked collagen caused by the over-expression lysyl oxidase (LOX) in heart tissue is the underlying cause of certain types of heart failure. This study has two aspects: the first is improving the analysis of hydrolyzed collagen from tissue (crosslinks) and the second is the analysis of crosslinked peptides. Our goal is to be able to detect and quantify crosslinks and correlate them to LOX activity, disease states, and effectiveness of treatment in mouse models. While the analysis of LOX activity is our primary interest, there are fundamental chemistry interests as well: we have reason to believe that these doubly and triply crosslinked peptides will have interesting gas phase ion behavior including unique fragmentation patterns.



- Hanson DA, Eyre DR 1996 J. Biol. Chem. 271, 26508